

CLAIMS

1. A method for producing a mutation in a particular region of DNA of a *P. haemolytica* genome:

isolating said region of the genome from *P. haemolytica*;

introducing a mutation into said region to form a mutated DNA region;

methylating said mutated DNA region with a methylating enzyme which inhibits endonuclease cleavage at a recognition sequence selected from the group consisting of 5'-GATGC-3' and 5'-GCATC-3', to form methylated DNA;

introducing said methylated DNA into a *P. haemolytica* cell to form transformants; and

screening said transformants for those which have said mutation in said region on chromosomal DNA of said *P. haemolytica* cell.

2. The method of claim 1 wherein said step of methylating is performed by passage of said DNA region through a methylating cell containing *PhaI* methylase.

3. The method of claim 1 wherein said step of methylating is performed by passage of said DNA region through a methylating cell containing *SfaNI* methylase.

4. The method of claim 1 wherein the step of methylating is performed *in vitro*.

5. The method of claim 1 wherein the methylating enzyme is *PhaI* methyltransferase.

6. The method of claim 1 wherein the methylating enzyme is *SfaNI* methyltransferase.

7. The method of claim 2 wherein said methylating cell is a *P. haemolytica* strain which contains no *PhaI* restriction endonuclease activity.

8. The method of claim 2 wherein said methylating cell is a bacterium other than *P. haemolytica* which contains a gene encoding *PhaI* methylase.

The method of claim 2 wherein said methylating cell is a bacterium other than *Streptococcus faecalis* which contains a gene encoding *Sfa*NI methylase.

10. The method of claim 1 wherein said methylated DNA is introduced into *P. haemolytica* on a plasmid containing a *P. haemolytica* 4.2 kb Str^R plasmid deposited at the ATCC as Accession No. ATCC 69499.

11. The method of claim 10 further comprising:

screening said transformants for loss of said 4.2 kb Str^R plasmid.

12. An isolated and purified gene encoding *Pha*I methyltransferase.

13. An isolated and purified gene encoding *Pha*I restriction endonuclease.

14. A preparation of *Pha*I methyltransferase free from *Pha*I restriction endonuclease.

15. A preparation of *Pha*I endonuclease free from *Pha*I methyltransferase.

16. The preparation of claim 14 which is free from all other *P. haemolytica* proteins.

17. The preparation of claim 15 which is free from all other *P. haemolytica* proteins.

18. A chimeric plasmid for unstable introduction of genetic material into *P. haemolytica* comprising two plasmids covalently linked to each other, wherein the first plasmid is a 4.2 kb Str^R plasmid of *P. haemolytica* deposited at the American Type Culture Collection as Accession No. ATCC 69499; and the second plasmid is a plasmid which cannot replicate in *P. haemolytica*.

19. The chimeric plasmid of claim 18 further comprising:
a region of the chromosome of *P. haemolytica* wherein said region harbors a mutation.

20. A *P. haemolytica* mutant made by the process of claim 1.

21. *P. haemolytica* strain NADC-D60aroA⁻, deposited at the ATCC as Accession No. ATCC 55518.

22. A *P. haemolytica* strain which harbors a mutation which abolishes expression of *Pha*I restriction endonuclease.

23. A vaccine comprising an attenuated, live, mutant of *P. haemolytica*, which comprises an *aroA* mutation.

24. The vaccine of claim 23 wherein said mutation is a non-reverting mutation.

25. The vaccine of claim 23 wherein said mutation is an insertion mutation.

26. The vaccine of claim 23 wherein said mutation is genetically linked to a selectable marker.

27. A method for producing a mutation in a particular region of DNA of a *P. haemolytica* genome:

isolating said region of the genome from *P. haemolytica*;

introducing a mutation into said region to form a mutated DNA region;

introducing said mutated DNA region into a *P. haemolytica* cell which does not express a *PhaI* restriction endonuclease, to form transformants; and screening said transformants for those which have said mutation in said region on chromosomal DNA of said *P. haemolytica* cell.

28. The method of claim 27 wherein said *P. haemolytica* cell which does not express a *PhaI* restriction endonuclease is a natural isolate.

29. The method of claim 27 wherein said *P. haemolytica* cell which does not express a *PhaI* restriction endonuclease is a mutant made by chemical mutagenesis.

30. The method of claim 27 wherein said *P. haemolytica* cell which does not express a *PhaI* restriction endonuclease is a mutant made by the process of claim 1.

31. A *P. haemolytica* mutant made by the process of claim 27.

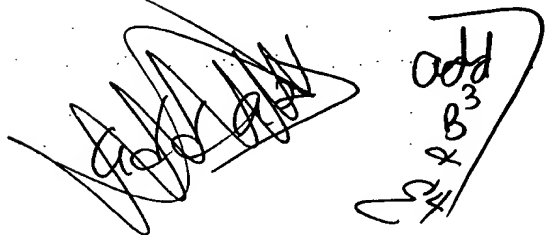
32. An isolated and purified *P. haemolytica* strain which has been genetically modified by the stable introduction of DNA.

33. The *P. haemolytica* strain of claim 32 wherein the introduced DNA has recombined with genomic DNA of *P. haemolytica*.

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